CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
KIH-2023 (Bispyribac - Sodium)

Chemical Code # 5749, Tolerance # 52841
Original date: April 11, 2001
Revised date: 5/23/01

I. DATA GAP STATUS

Combined, rat:	No data gap, acceptable study, no adverse effect.
Chronic toxicity, dog:	No data gap, acceptable study, no adverse effect
Oncogenicity, mouse:	No data gap, acceptable study, no adverse effect
Reproduction, rat:	No data gap, acceptable study, no adverse effect
Teratology, rat:	No data gap, acceptable study, no adverse effect
Teratology, rabbit:	No data gap, acceptable study, no adverse effect
Gene mutation:	No data gap, acceptable study, no adverse effect
Chromosome effects:	No data gap, acceptable study, no adverse effect
DNA damage:	No data gap, acceptable study, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T186463.doc
Original: M. Silva, 4/11/01
Revised: 5/23/01

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 072 173422 "Chronic Feeding and Oncogenicity Study in Rats with KIH-2023 Technical," (Inoue, H.; Biosafety Research Center, Foods, Drugs and Pesticides, (An-Pyo Center), Shizuoka-ken, Japan; Laboratory Project ID#: 3127 10/2/95). KIH-2023 technical (97.2% pure) was fed in diet to F344 Fischer (SPF) rats (98/sex/dose, with 10/sex/dose to be terminated at weeks 13, 26, 52, 78 & the remaining survivors at week 104) at 0, 20, 200, 3500 (M), 5000 (F) and 7000 (M), 10000 (F) ppm. Mean test substance intakes: 1.1, 10.9, 195 and 405 mg/kg/day for males and 1.4, 13.8, 352 and 715 mg/kg/day for females. SYSTEMIC NOEL = 200 ppm (Male mortality at 7000 ppm was increased along with wasting (≥ 3500 ppm), piloerection, dirty hair, pallor/auricles, abdominal distention and decreased spontaneous motor activity. Both sexes showed decreased body weights at ≥ 3500 ppm (M) and at ≥ 5000 ppm (F). Males showed decreased food consumption and food efficiency at ≥ 3500 ppm and females at ≥ 5000 ppm, sporadically, throughout the study. Both sexes had numerous hematological and clinical chemistry effects at ≥ 3500 ppm (M) and ≥ 5000 ppm (F). Males had increased urine volume and sodium at 7000 ppm and calcium at ≥ 3500 ppm, with decreased potassium at ≥ 3500. Females had decreased pH in urine and decreased sodium and potassium at 10000 ppm. Males had increased absolute and relative liver weights at 7000 ppm, sporadic decreases in heart weights and increases in spleen weights and relative adrenal, kidney and brain weights at 7000 ppm. Females had increased absolute kidney and ovary weights, relative liver and brain weight and relative kidney weights at 10000 ppm. There were numerous gross and histopathological effects in liver and choledochus at ≥ 3500 ppm (M) and ≥ 5000 ppm (F). There was testicular atrophy and proliferative ducts in pancreas at ≥ 3500 ppm (M) and ≥ 5000 ppm (F).ONCOGENICITY NOEL > 7000 ppm (M) & 10000 ppm (F) (There were no treatment-related effects at any dose.) Acceptable. No adverse effect. M. Silva, 2/28/01.

CHRONIC TOXICITY, RAT

095 173445 "Mechanistic Study for the Effects on Urinary Bladder and Ductus choledochus in Prolonged Dietary Administration of KIH-2023 Technical in Rats," (Inoue, H.; Biosafety Research Center, Foods, Drugs and Pesticides, (An-Pyo Center), Japan; Laboratory Project ID#: 2630; 7/1/94). KIH-2023 technical (97.2% pure) was fed in diet to Fischer F344 (SPF) rats (25/sex/dose/sacrifice) at 0 and 7000 ppm (males)/10000 ppm (females) with 5 sacrificed at 4, 13 and 52 weeks and at week 26 (+ 13 week recovery) and week 17 (+ 4 week recovery). At 0 and 200 ppm, 5/sex were sacrificed at 4, 13 and 52 weeks. Doses were equivalent to12.3 and 447 mg/kg/day for males and 13.8 and 724 mg/kg/day for females. Another group of rats (15/sex) were treated at 200 ppm (M: 12.3 mg/kg/day, F: 13.8 mg/kg/day) and sacrificed at week 52. Male body weight at ≥ 200 ppm and body weight gain at 7000 ppm were decreased to 52 weeks. Female body weights were decreased at 10000 ppm. Recovery body weight gain in females at 10000 ppm was increased weeks 13-26. Males had increased LAP, γ-GTP, ALP, total bilirubin, 5'nucleotidase and total bile acid at 7000 ppm at weeks 4 and 13. These effects were reversed during the recovery period. Females had increased γ-GTP at 10000 ppm at week 4. Both sexes showed decreased food consumption at ≥ 200 ppm (52 weeks) and males at 7000 ppm showed decreased food efficiency. Males had dilated lumen in choledochus at 7000 ppm (52 weeks). Males and females had black patch/zone in pituitary gland (52 weeks) at 7000 and 10000 ppm, respectively. 1/5 Males had transitional cell hyperplasia of the urinary bladder at 7000 ppm (4 weeks). Males at 13 weeks, 17 weeks, 26 weeks and 52 weeks had choledochus muscular hypertrophy (7000 ppm). Male liver at 52 weeks had accumulation of macrophage, bile duct hyperplasia, fibrosis and proliferative ducts at 7000 ppm. Choledochus had lymphangiectasis (1/5), dilatation, hyperplastic epithelium and fibrosis in males at 7000 ppm. Females at 17 weeks, 26 weeks and 52 weeks had choledochus muscular hypertrophy at 10000 ppm. Females at 17 weeks and 26 weeks had urinary bladder lymphocytic infiltration at 10000 ppm. Female livers at 52 weeks had granulation, fibrosis and proliferative ducts at 10000 ppm. Males had increased PCNA-positive liver cell nuclei at 7000 ppm at week 52. Females had decreased PCNA-positive ductus choledochus nuclei at 10000 ppm at weeks 17 and 26. SYSTEMIC NOEL = 200 ppm (histopathology of liver, choledochus and urinary bladder). Possible adverse effect. M. Silva, 3/29/01.

CHRONIC TOXICITY, MOUSE

094 173444 "Mechanistic Study for the Effects on Gall Bladder in Prolonged Dietary Administration of KIH2023 Technical in Mice," (Inoue, H.; Biosafety Research Center, Foods, Drugs and Pesticides, (An-Pyo Center), Shizuoka, Japan; Laboratory Project ID#: 2629; 10/31/94). KIH-2023 technical (98.4% pure) was fed in diet to B6C3F1 (SPF) mice (20/sex/dose) at 0 and 5000 ppm and (12/sex/dose) at 100 ppm. Mice were sacrificed at weeks: 52, 26 + 13-week recovery, 17 (+ 4 week recovery), 13 and 4 (0 & 5000 ppm) and at weeks: 52, 13 and 4 (100 ppm). SYSTEMIC NOEL = 100 ppm (Females intermittently showed significantly decreased body weights at 5000 ppm + 13-week recovery. There were decreased levels of total bilirubin in both sexes receiving 5000 ppm. Both sexes showed intermittently increased food consumption at 5000 ppm + 13-week recovery. Food efficiency was increased primarily at 5000 ppm in both sexes (later decreased). 1 of 4 males at 5000 ppm had white patch/zone on the liver (52 week sacrifice). 1 of 4 females (each) and 2/4 males at 5000 ppm had anisonucleosis (4 & 13 week sacrifice-F & 13 week + recovery--M). This was increased in males at 5000 ppm at 52 week sacrifice (4/4, compared with 2/4 each in control & 100 ppm). 2 of 4 females (13 week sacrifice) and 1 of 4 males (13 week + recovery) at 5000 ppm had gall bladder dilatation. At 52 week sacrifice, 1 of 4 females each had gall bladder dilatation at 100 and 5000 ppm. 2 of 4 males at 5000 ppm had giant cells in liver at 52 week sacrifice.) No adverse effect. Supplemental data. M. Silva, 3/28/01.

CHRONIC TOXICITY, DOG

** 072 173513 "KIH-2023 Technical: Fifty-Two Week Oral Toxicity Study in Beagle Dogs," (Hashiguchi, J.; Biosafety Research Center, Foods, Drugs and Pesticides, (An-Pyo Center), Shizuoka, Japan; Laboratory Project ID#: 2618; Original report date: 2/3/94; Amendment date: 8/26/94). KIH-2023 technical (98.7% pure) was administered by capsule (4/day) to Beagle dogs (4/sex/dose) at 0, 10, 100 and 750 mg/kg/day for 52 weeks. (A transitional bodyweight decrease was observed in 1 female at 750 mg/kg/day (#2303). A transitional decrease in food consumption was observed in 1 female at 750 mg/kg/day (#2303). Females, at 750 mg/kg showed lower % of large unstained cells (LUC). 5'Nucleotidase levels in females at $\geq$ 100 mg/kg/day and blood urea nitrogen in females at 750 mg/kg were lower than controls. Both sexes at 750 mg/kg showed an increase in urine cast. The absolute and relative (body) weights of liver were higher in males at 750 mg/kg. The absolute thyroid weights in males were increased at 750 mg/kg. No mortality was reported. Clinical signs (salivation, vomiting and loose stool) were seen at 750 mg/kg/day in both sexes.) SYSTEMIC NOEL = 10 mg/kg/day (Both sexes had liver bile duct hyperplasia at $\geq$ 100 mg/kg. Epithelial hyperplasia of the choledochus occurred in males at 750 mg/kg.) Acceptable. No adverse effect. M. Silva, 3/15/01.

ONCOGENICITY, MOUSE

Subchronic Studies:

064 173514 "KIH-2023: A Four-Week Dietary Range-Finding Study in Mice," (Inoue, H.; Biosafety Research Center, Foods, Drugs and Pesticides, (An-Pyo Center), Shizuoka-ken, Japan; Laboratory Project ID#: 1693; 9/5/91). KIH-2023 technical (96.3% pure) was fed in diet to B6C3F1 (SPF) mice (10/sex/dose) at 0, 10, 50, 500, 7000 and 30000 ppm for 4 weeks. NOEL = 50 ppm, based on male increased thymic karyorrhexia and decreased spleen weights and female decreased food efficiency at > 500 ppm. Other effects: Bodyweights and bodyweight gains were significantly decreased in females at $\geq$ 7000 ppm and in males at 30000 ppm. Males had decreased food efficiency at 30000 ppm. Both sexes had significantly decreased absolute brain, kidney, spleen and testes (male) weights at 30000 ppm. Males showed decreased relative kidney weights and increased brain weights at 30000 ppm. Females had significantly increased relative brain ($\geq$ 7000 ppm) and decreased spleen weights (30000 ppm). Both sexes had relative liver weight increases at 30000 ppm. Males had increased cecal dilated lumen at $\geq$ 7000 ppm. At 30000 ppm, males showed increased green gall bladder, black liver and white patches/zones on the liver. Females at 30000

ppm showed increased green gall bladder, black gall bladder, dilated cecal lumen and small uteri. Both sexes had increased liver and gall bladder effects at ≥ 7000 ppm. Females had increased stomach edema and thymic karyorrhexia at ≥ 7000 ppm. No adverse effect. These data are supplemental. M. Silva, 2/14/01.

Oncogenicity Study:

** 065 173515 "Oncogenicity Study in Mice with KIH-2023 Technical," (Inoue, H.; Biosafety Research Center, Foods, Drugs and Pesticides, (An-Pyo Center), Shizuoka-ken, Japan; Laboratory Project ID#: 3126; 10/2/95). KIH-2023 technical (98.4% pure) was fed in diet to B6C3F1 (SPF) mice (80/sex/dose, with 10/sex/dose to be terminated at weeks 26, 52, 78 & the remaining survivors at week 104) at 0, 10, 100, 2500 and 5000 ppm (equivalent to 1.4, 14.1, 353 and 729 mg/kg/day in males and 1.7, 7.4 448 and 903 mg/kg/day in females). (Both sexes showed decreased bodyweights and decreased bodyweight gain, intermittently at ≥ 2500 ppm throughout the study. Males showed decreased food efficiency at 5000 ppm. Both sexes showed intermittent fluctuations in HCT, RBC, MCV, MCH, MCHC, neutrophils and lymphocytes, primarily at ≥ 2500 ppm. Absolute liver weights were decreased in females at 5000 ppm. At 52 weeks, relative (to bodyweight) brain, heart and kidney weights were increased in females at 5000 ppm. At 104 weeks, males had increased relative testes weights and females had increased relative brain and kidney weights at 5000 ppm. Decreased relative (to brain) liver weights occurred in females at 5000 ppm throughout the study.) SYSTEMIC NOEL = 100 ppm There were other non-neoplastic effects in liver (centrilobular swelling of hepatocytes and appearance of giant cells) in males at 2500 and 5000 ppm. ONCOGENICITY NOEL > 5000 ppm (There were no treatment-related effects at any dose.) No adverse effect. Acceptable. (M. Silva, 2/20/01).

REPRODUCTION, RAT

Range-Finding:

070 173520 "Reproduction Range-Finding Study in Mated Rats with KIH-2023," (York, R.G.; IRDC, Mattawan, MI; Laboratory Project #: 442-040; 10/27/92). KIH-2023 technical (98.2% pure; sodium 2,6-bis[(4, 6-dimethoxypyrimidin-2-yl)oxy]benzoate) was fed in diet to Charles River Crl:CD® VAF/Plus® rats (8/sex/dose) at 0, 100, 1000, 10000 and 20000 ppm from 28 days prior to F0 mating through F1 weaning at day 21. Actual doses were for Males: 8.1, 82.0, 830.1 and 1620.7 mg/kg/day and for Females: 8.1, 78.5, 789.3 and 1589.8 mg/kg/day. Systemic NOEL = 1000 ppm (Maternal bodyweight gains during gestation days 14-20 and 0-20 were statistically significantly decreased at 20000 ppm. Maternal bodyweight gain during lactation was increased in dams at ≥ 10000 ppm. Males at 20000 ppm had significantly decreased food consumption during study week 1. Reproductive NOEL = 1000 ppm (The numbers of implantation sites and delivered offspring were decreased at ≥ 10000 ppm, compared to control.) Pup NOEL = 1000 ppm (Three and 2 pups at 10000 and 20000 ppm died between lactation days 0 and 3.) No adverse effect indicated. These data are supplemental. M. Silva, 3/6/01

Definitive Study:

** 071 173521 "Two Generation Reproduction/Fertility Study in Rats with KIH-2023," (Schardein, J.L.; IRDC, Mattawan, MI; Laboratory Project #: 442-041; 6/12/94). KIH-2023 technical (98.2% pure; sodium 2,6-bis[(4, 6-dimethoxypyrimidin-2-yl)oxy]benzoate) was fed in diet to Charles River Crl:CD® VAF/Plus® rats (26/sex/dose) at 0, 20, 1000 and 10000 ppm from age 43 days and for 56 days prior to F0 mating through F1 weaning. From the F1 litters 26/sex/dose, were selected and were treated beginning at age 22 days for 105 days prior to mating, through weaning of F2 pups. There was an increase in anogenital staining in F0 females at 10000 ppm. Bodyweights of F1 parental males were significantly decreased from week 4 (beginning of F1 generation) at 10000 ppm. F1 female bodyweights were significantly decreased from week 4. F1 females had significantly decreased bodyweights during gestation days 15-20 and during lactation days 0-21 at 10000 ppm. F1 male

food consumption was sporadically, significantly decreased weeks 4-20 at 10000 ppm. Systemic NOEL (M/F) = 20 ppm (Histological changes in the choledochus (hyperplastic epithelium) and liver (bile duct hyperplasia) were noted in both F0 and F1 generation at the mid and high dose.) Reproductive NOEL > 10000 ppm (There were no treatment-related effects at any dose.) Pup NOEL = 1000 ppm (F1 and F2 pup weights were significantly decreased on day 1 (both sexes) and through day 21 (males).) No adverse effect. Acceptable. M. Silva, 3/6/01

TERATOLOGY, RAT

Rangefinding Study:

066 173516 "Range-finding Teratology Study in Rats with KIH-2023," (Breslin, W.J.; MPI Research (formerly IRDC), Mattawan, MI; Laboratory Project ID#: 442-036; Original Report Date: 6/20/90; Amendment Date: 5/6/98). KIH-2023 technical (96.3% pure) was administered by gavage to mated Charles River Crl:CD VAF/Plus® rats (6/dose) at 0, 100, 250, 500 and 1000 mg/kg/day during days 6-15 of gestation. Maternal NOEL > 1000 mg/kg/day (There were no significant, treatment-related effects at any dose.) Developmental NOEL > 1000 mg/kg (There were no treatment-related effects at any dose.) No adverse effect. These data are supplemental. M. Silva, 3/16/01.

Definitive Study:

** 067 173517 "Teratology Study in Rats with KIH-2023," (York, R.G.; IRDC, Mattawan, MI; Laboratory Project ID#: 442-038; 4/25/91). KIH-2023 technical (95.2% pure) was administered by gavage to mated Charles River Crl:CD VAF/Plus® rats (25/dose) at 0, 100, 300 and 1000 mg/kg/day (limit test) during days 6-15 of gestation. Maternal NOEL = 300 mg/kg/day (Anogenital staining was observed at 1000 mg/kg/day. These observations were of negligible toxicological significance.) Developmental NOEL > 1000 mg/kg/day (There were no treatment-related developmental effects at any dose.) No adverse effect. Acceptable. M. Silva, 3/22/01.

TERATOLOGY, RABBIT

Rangefinding Study:

068 173518 "KIH-2023: Preliminary Teratology Study in the Rabbit," (Kawanishi, H.; The Imamichi Institute for Animal Reproduction, Ibaraki, Japan; Laboratory Project ID#: 253-A; 8/31/90). KIH-2023 technical (95.2% pure) was administered by gavage to mated Japanese White rabbits (JW-NIBS (7/dose) at 0, 75, 150, 300 and 500 mg/kg/day during days 6-18 of gestation. Maternal NOEL = 75 mg/kg/day, based on premature delivery at ≥ 150 mg/kg/day. (1/7 (control) and 5/7 (500 mg/kg) were found dead. The control and 1 death at 500 mg/kg were due to dosing error. Immature delivery occurred in 1/6 at 150 mg/kg, 2/6 at 300 mg/kg and 1/7 at 500 mg/kg. Sedation was observed in 2/6 dams at 300 mg/kg and 6/7 dams at 500 mg/kg. Decreased body weight gain occurred at ≥ 300 mg/kg. Food intake was significantly decreased days 9-18 of gestation at ≥ 300 mg/kg. Dams dying prematurely at 500 mg/kg showed gastric membrane hemorrhage and atrophy of spleen (3/5). In dams sacrificed due to premature delivery, dense spots on lungs (F18 at 150 mg/kg), gastric hemorrhage (F25 & F26, 300 mg/kg) and sclerosis of liver (F34 at 500 mg/kg) were observed.) Developmental NOEL > 500 mg/kg/day (There were no treatment-related developmental effects at any dose.) No adverse effects indicated. These data are supplemental. M. Silva, 3/26/01.

Definitive Study:

** 069 173519 "KIH-2023: Teratology Study in Rabbits," (Kawanishi, H.; The Imamichi Institute for Animal Reproduction, Ibaraki, Japan; Laboratory Project ID#: 253-B; 10/5/92). KIH-2023 technical (98.4% pure) was administered by gavage to mated Japanese White rabbits (JW-NIBS (20/dose) at 0, 30, 100 and 300 mg/kg/day during days 6-18 of gestation. Maternal NOEL = 100 mg/kg/day (1/20 (300 mg/kg) died on day 25 of gestation.) Premature delivery was found at ≥ 100 mg/kg (1 in each group). Soft feces were observed (not significant) in 2 control and 6 at each treatment dose. Developmental NOEL = 100 mg/kg/day (There was one fetus at 300 mg/kg with cleft palate.) This

study is complete and acceptable with no adverse effect. M. Silva, 3/27/01.

GENE MUTATION

** 085 173435 "Mutagenicity Test on KIH-2023: In the Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay," (Lawlor, T.E, DaCosta, K..; Hazleton Laboratories America, Inc., Kensington, Maryland; Laboratory Project #: 12130-0-409R; 8/14/90). KIH-2023 technical (95.2% pure) was used with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 333, 667, 1000, 3330, 6670 and 10000 ug/plate (3 plates/dose), both with and without S9 microsomal activation. A preliminary rangefinding test was performed to optimize treatment at 0, 10, 33.3, 66.7, 100, 333, 667, 1000, 3330, 6670 and 10000 ug/plate performed with TA100 & WP2uvrA$^-$, +/- S9 (1 plate/dose/strain). The dose rangefinding study (TA100 & WP2uvrA$^-$, +/- S9) showed that no cytotoxicity was evident at any dose, either with or without S9. Two independent tests were performed for the definitive study. There were no increases in revertant colonies with the *S. typhimurium* strains or with WP2uvrA$^-$, either with or without S9. Acceptable. No adverse effect. M. Silva, 4/3/01.

Metabolite of KIH-2023:

** 087 173437 "Mutagenicity Test on Me_2-BA: In the Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay," (Lawlor, T.E; Hazleton Laboratories America, Inc., Kensington, Maryland; Laboratory Project #: 12517-0-409R; 3/7/91). Me_2-BA technical (99.8% pure) was used with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 100, 333, 667, 1000, 3330 and 5000 ug/plate (3 plates/dose), both with and without S9 microsomal activation. A preliminary rangefinding test was performed to optimize treatment at 0, 6.67, 10, 33.3, 66.7, 100, 333, 667, 1000, 3330 and 5000 ug/plate performed with TA100 & WP2uvrA$^-$, +/- S9 (1 plate/dose/strain). Two independent tests were performed for the definitive study. The dose rangefinding study (TA100 & WP2uvrA$^-$, +/- S9) showed that no cytotoxicity was evident at any dose, either with or without S9. There were no increases in revertant colonies with the *S. typhimurium* strains or with WP2uvrA$^-$, either with or without S9. Acceptable (for a metabolite). No adverse effect. M. Silva, 4/3/01.

** 082 173432 "Mutagenicity Test on BX-180: In the Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay," (Lawlor, T.E.; Hazleton Laboratories America, Inc., Kensington, Maryland; Laboratory Project #: 12516-0-409R; 3/7/91). BX-180 technical ([2-(4,6-dimethoxy-pyrimidin-yl)oxy-6-hydroxybenzoic acid; 100% pure) was used with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 100, 333, 667, 1000, 3330 and 6670 ug/plate (3 plates/dose), both with and without S9 microsomal activation. A preliminary rangefinding test was performed to optimize treatment at 0, 10, 33.3, 66.7, 100, 333, 667, 1000, 3330, 6670 and 10000 ug/plate performed with TA100 & WP2uvrA$^-$, +/- S9 (1 plate/dose/strain). The dose rangefinding study (TA100 & WP2uvrA$^-$, +/- S9) showed that cytotoxicity was evident with TA100 at $\geq$ 6670 ug/plate (+S9) and $\geq$ 3330 ug/plate (-S9) and with WP2uvrA$^-$ at $\geq$ 6670 ug/plate (-S9). The results of this test were used to select doses for the definitive study. Two independent tests were performed for the definitive study. There were no increases in revertant colonies with the *S. typhimurium* strains or with WP2uvrA$^-$, either with or without S9. Acceptable (for a metabolite). No adverse effect. M. Silva, 4/3/01.

** 074 173424 "Mutagenicity Test on DesMe-2023: In the Salmonella-Escherichia Coli/Mammalian-Microsome Reverse Mutation Assay," (Lawlor, T.E.; Hazleton Laboratories America, Inc., Kensington, Maryland; Laboratory Project #: 12515-0-409R; 3/7/91). DesMe-2023 technical (80.1% pure) was used with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *Escherichia coli* strain WP2uvrA$^-$ at 6 doses ranging from 66.7 to 6670 ug/plate (3 plates/dose), both with and without S9 microsomal activation. A preliminary rangefinding test was performed to optimize treatment. Two independent tests were performed for the definitive study. The dose rangefinding

study (TA100 & WP2uvrA$^-$, +/- S9) showed that cytotoxicity was evident with TA100 at $\geq$ 3330 ug/plate (+S9) and $\geq$ 333 ug/plate (-S9) and with WP2uvrA$^-$ at $\geq$ 3330 ug/plate (+/- S9). The results of this test were used to select 6 doses for the definitive study. In the initial mutagenicity study, TA98 was contaminated and the entire TA98 assay was repeated. There were no increases in revertant colonies with the *S. typhimurium* strains or with WP2uvrA$^-$, either with or without S9. Acceptable (for a metabolite). No adverse effect. M. Silva, 4/3/01.

** 075 173425 "2, 4-Dihydroxy-6-Methoxy Pyrimidine: Assessment of Mutagenic Potential in Amino-Acid Auxotrophs of *Salmonella typhimurium* and *Escherichia Coli* (The Ames Test)," (May, K.; Pharmaco LSR Ltd., Suffolk, England; Laboratory Project #: 95/KC1170/0198; 3/21/95). DMP (2, 4-Dihydroxy-6-Methoxy Pyrimidine; 99.1% pure) was used with *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1538 and TA1535 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 50, 158, 500, 1580 and 5000 ug/plate (plates/dose/strain, 2 independent assays) after a preliminary toxicity test with TA98 and WP2uvrA$^-$ was performed at 0, 5, 50, 500 and 5000 ug/plate (3 plates/dose/ strain). Assays were performed both with and without S9 microsomal activation. There was no increase in revertant colonies, compared with control. The positive controls functioned as expected. Acceptable (for a metabolite). No adverse effect. M. Silva, 4/3/01.

** 077 173427 "KIH-2023-I-1: Six Strain Reverse Mutation Assay "Ames Test" Using *Salmonella Typhimurium* and *Escherichia Coli*," (Thompson, P.W.; Safepharm laboratories Limited, Derby DE1 2BT, UK; Project #: 131/201R; 8/20/92). KIH-2023-I-1 technical (97.6% pure; metabolite) was used with *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1538 and TA1535 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 312.5, 625, 1250, 2500 and 5000 ug/plate (3 plates/dose/strain) and at 0, 8, 40, 200, 1000 and 5000 ug/plate (in triplicate) after a preliminary toxicity test with tester strains TA100 and WP2urvA$^-$ at 0, 312.5, 625, 1250, 2500 and 5000 ug/plate. Assays were performed both with and without S9 microsomal activation. KIH-2023-I-1 caused no reduction in the growth of the bacterial lawn at any dose level either with or without S9 (tested to the maximum recommended dose of 5000 ug/plate). There was no increase in revertant colonies, compared with control. The positive controls functioned as expected. Acceptable (for a metabolite). No adverse effect. M. Silva, 4/3/01.

** 080 173430 "KIH-2023-M-8-Na: Assessment of Mutagenic Potential in Amino-Acid Auxotrophs of *Salmonella typhimurium* and *Escherichia Coli* (The Ames Test)," (May, K.; Pharmaco LSR Ltd., Suffolk, England; Laboratory Project #: 95/KCI158/0005; 3/13/95). KIH-2023-M-8-Na technical (87.9% pure, hydroxy derivative) was used with *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1538 and TA1535 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 50, 158, 500, 1580 and 5000 ug/plate (3 plates/dose/strain, 2 independent assays) after a preliminary toxicity test with TA98 and WP2uvrA$^-$ was performed at 0, 5, 50, 500 and 5000 ug/plate (3 plates/dose/ strain). Assays were performed both with and without S9 microsomal activation. There was no increase in revertant colonies, compared with control. The positive controls functioned as expected. Inhibition of bacterial growth, occurred in all Salmonella strains following exposure to KIH-2023-M-8-Na at 5000 ug/plate. Acceptable (for a hydroxy derivative). No adverse effect. M. Silva, 4/3/01.

** 081 173431 "KIH-2023-M-9-Na: Assessment of Mutagenic Potential in Amino-Acid Auxotrophs of *Salmonella typhimurium* and *Escherichia Coli* (The Ames Test)," (May, K.; Pharmaco LSR Ltd., Suffolk, England; Laboratory Project #: 95/KCI160/0006; 4/3/95). KIH-2023-M-9-Na technical (88.1% pure, metabolite) was used with *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1538 and TA1535 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 50, 158, 500, 1580 and 5000 ug/plate (3 plates/dose/strain, 2 independent assays) after a preliminary toxicity test with TA98 and WP2uvrA$^-$ was performed at 0, 2.5, 25, 250 and 2500 ug/plate and at 5, 50, 500 and 5000 ug/plate (1 plate/dose/strain). Assays were performed both with and without S9 microsomal activation. There was no increase in revertant colonies, compared with control. The positive controls functioned as expected. Acceptable (for a metabolite). No adverse effect. M. Silva, 4/6/01.

Impurity of KIH-2023:

** 078 173428 "KIH-2023-I-2: Reverse Mutation Assay "Ames Test" Using *Salmonella Typhimurium* and *Escherichia Coli*," (Thompson, P.W.; Safepharm Laboratories Limited, Derby DE1 2BT, UK; Project #: 131/342; 12/7/95). KIH-2023-I-2 technical (97.1% pure; impurity of KIH-2023 Technical) was used with *Salmonella typhimurium* strains TA98, TA100, TA1537 and TA1535 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 50, 150, 500, 1500 and 5000 ug/plate (3 plates/dose/strain; 2 experiments) after a preliminary toxicity test with all tester strains at 0, 50, 150, 500, 1500 and 5000 ug/plate (1 plate/dose/ strain). Assays were performed both with and without S9 microsomal activation. KIH-2023-I-2 caused no reduction in the growth of the bacterial lawn at any dose level either with or without S9 (tested to the maximum recommended dose of 5000 ug/plate). There was no increase in revertant colonies, compared with control. The positive controls functioned as expected. Acceptable (an impurity of KIH-2023 Technical). No adverse effect. M. Silva, 4/5/01.

** 079 173429 "KIH-2023-I-4: Reverse Mutation Assay "Ames Test" Using *Salmonella Typhimurium* and *Escherichia Coli*," (Thompson, P.W.; Safepharm laboratories Limited, Derby DE1 2BT, UK; Project #: 131/344; 12/7/95). KIH-2023-I-4 technical (99.9% pure; impurity of KIH-2023 technical) was used with *Salmonella typhimurium* strains TA98, TA100, TA1537 and TA1535 and *Escherichia coli* strain WP2uvrA$^-$ at 0, 50, 150, 500, 1500 and 5000 ug/plate (3 plates/dose/strain; 2 experiments) after a preliminary toxicity test with tester strains TA100 and WP2uvrA- at 0, 50, 150, 500, 1500 and 5000 ug/plate. Assays were performed both with and without S9 microsomal activation. KIH-2023-I-4 caused no reduction in the growth of the bacterial lawn at any dose level either with or without S9 (tested to the maximum recommended dose of 5000 ug/plate). There was no increase in revertant colonies, compared with control. The positive controls functioned as expected. Acceptable (for an impurity of KIH-2023 technical). No adverse effect. M. Silva, 4/5/01.

CHROMOSOME EFFECTS

** 083 173433 "Mutagenicity Test on KIH-2023: In an *in Vitro* Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells with Multiple Harvests," (Murli, H.; Hazleton Laboratories America Inc., Kensington, Maryland; Laboratory Project #: 12130-0-437J; Genetic assay #: 12130; 8/16/90). KIH-2320 technical (95.2% pure) was used on CHO cells at 0, 166, 499, 1660 and 4990 (+ & - S9) in an initial rangefinding test, then at 0, 1500, 2000, 3000 and 4000 ug/ml (no S9, 2 slides/dose) or 0, 1250, 2500, 3750 and 5010 (or 5000) ug/ml (+S9, 2 hours). Harvest times without S9 were 10 hours (7.25 hour exposure), 20 hours (17.2 hour exposure) and 30 hours (29.3 hour exposure). Harvest times with S9 were 10 and 20 hours (2 hour exposure). Results showed that in the 30 hour harvest (-S9) at $\geq$ 3000 ug/ml, cytotoxicity was so high that there were only 80 cells from replicate culture available for accurate evaluation. In the 20 hour harvest (-S9), cytotoxicity at 4000 ug/ml (high dose) was observed, such that only 40 and 60 cells/slide could be counted. In addition, the % of cells with aberrations was increased at this dose, however these values were not statistically significant (0% versus 2%). There was no increase in chromosomal aberration with S9 at the 10 or 20 hour harvest. No adverse effect. Acceptable. M. Silva, 4/10/01.

DNA DAMAGE

073 173423 "Dose Rangefinding Study for in Vivo Murine Micronucleus Assay on KIH-2023," (Murli, H.; Hazleton Washington Inc., Rockville, Maryland; Laboratory Project #: 14337-0-459PO; Genetic assay #: 14337; 5/14/91). KIH-2320 technical (94.5% pure) was administered once by gavage to ICR mice (3/sex/dose) at 0 (0.5% carboxymethyl cellulose), 500, 1625, 2750, 3875 and 5000 mg/kg. All animals were examined after dosing and daily throughout the duration of the study (3 days) for toxic effects and mortality. No toxic effects were observed immediately after dosing, but within 24 hours, 1 male (#4191) at 2750 mg/kg was found dead. The MTD was gauged to be >5000 mg/kg and the results were sufficient to select doses for the definitive mouse micronucleus assay. Supplemental data. No adverse effect. M. Silva, 4/9/01.

** 086 173436 "Mutagenicity Test on KIH-2023: *in Vivo* Micronucleus Assay," (Murli, H.; Hazleton Washington Inc., Kensington, Maryland; Laboratory Project #: 14337-0-455PO; Genetic assay #: 14337; 7/29/91). KIH-2320 technical (94.5% pure) was administered once by gavage to ICR mice (5/sex/dose/time point) at 0 (0.5% carboxymethyl cellulose; 20 ml/kg), 1250, 2500 and 5000 mg/kg. A secondary dose group at 5000 mg/kg was maintained to replace animals that died. The positive control was cyclophosphamide (80 mg/kg). Animals were euthanized at 24, 48 and 72 hours post-dosing for extraction of bone marrow (vehicle and positive controls at 24 hours only). Micronuclei were assessed on slides (1000 polychromatic nuclei/slide). Approximately 4 hours after dosing, 4 males (#4496, 4509, 4415, 4465) and 3 females (#4495, 4479, 4453) at 5000 mg/kg were found dead. Prior to the 24 hour harvest, 1 male (#4506) was prostrate, experiencing tremors, and 1 female (#4430) was languid with squinted eyes (died within 7 hours) at 5000 mg/kg. The following morning, 3 males (#4639, 4625, 4552) and 1 female (#4580) at 5000 mg/kg were found dead and 1 female had previously been languid (#4580). On the last day, 1 female (#4563) at 5000 mg/kg was found dead. All animals at 2500 and 1250 mg/kg appeared normal. KIH-2023 did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this study. No adverse effect. Acceptable. M. Silva, 4/9/01.

** 084 173434 "Mutagenicity Test on KIH-2023: In the *in Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay With a Confirmatory Assay," (McKeon, M.E.; Hazleton Laboratories America, Inc., Kensington, Maryland; Laboratory Project #: 12130-0-447R; Genetic assay #: 12130; 8/10/90). KIH-2320 technical (95.2% pure) was used on primary hepatocytes (from adult male Fischer 344 rat) at 0, 0.05 to 5000 ug/ml in the presence of 10uCi/ml ^{3}HTdr (47 Ci/mmole) in a rangefinding study. Subsequently, in Trial 1, 15 treatments from 0.5 to 5000 ug/ml (5 culture dishes/dose; 2 cultures for cytotoxicity/dose; 3 slides (50 cells/slide)/dose) were initiated to assess unscheduled DNA synthesis. A second trial was initiated to clarify and confirm the lack of significant activity. KIH-2023 was highly toxic at 1000 ug/ml and above. No evidence of treatment-related UDS was observed. The positive controls functioned as expected. No adverse effect. Acceptable. M. Silva, 4/9/01.

**** 076 173426** "KIH-2023: Bacterial DNA Repair Assay," (Jones, E.; Huntingdon Research Centre, Ltd., Cambridgeshire, England; 12/17/97; Laboratory Project #: KCI 65/941552). KIH-2023 technical (95.7% pure) was used with *Bacillus subtilis* strains H17 rec^{+} (repair proficient) and M45 rec^{-} (repair deficient) in spot tests (toxicity/inhibition) at 0 (DMSO), 50, 150, 500, 1500 and 5000 ug/disk (1 plate/dose, both with & without S9 metabolic activation). At these same doses, KIH-2023 was also used in 2 independent differential killing assays with H17 and M45 (1 plate/dose/trial, + & - S9). Positive controls were 2-aminofluorene and 2-aminoanthracene (+S9) and AF-2 (-S9). Negative controls were kanamycin and streptomycin. SPOT TEST: KIH-2023 did not inhibit the growth of either tester strain, indicated by the lack of zones present on the nutrient agar plates. Therefore, a differential killing assay was performed. DIFFERENTIAL KILLING ASSAY: There was a substantial difference in toxicity (not dose-related) between the 2 strains in both the first and second differential killing assays at $\geq$ 50 ug/ml by cell survival/colony counts. Therefore, when KIH-2023 was tested up to 5000 ug/ml in DMSO, there was damage to bacterial DNA.
Acceptable. Possible adverse effect. M. Silva, 4/3/01.

Conclusion: Although there was a slight increase in differential killing at $\geq$ 50 ug/ml in the rec+/- assay, it appears that in all other mutagenicity assays and in *in vivo* studies that there is no indication of significant genetic damage. There were sufficient numbers of studies performed to adequately test these effects on DNA. Therefore, over all there will be no adverse effect flagged for DNA damage. However, it will be noted that study **076 173426** "KIH-2023: Bacterial DNA Repair Assay," Jones, E., was positive for DNA damage.

SUBCHRONIC STUDIES

(Oral)

056; 173506; "KIH-2023 Technical: Subchronic Toxicity Study in Rats" (Inoue, H., Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shioshinden, Shizuoka-ken, Japan, Laboratory Project Identification: 1943, 9/5/91). 821. KIH-2023 Technical (Lot No. G35-04, purity = 95.2%) was admixed to the diet at dose levels of 0 (basal diet only), 100, 1000, 10000, or 20000 ppm (for males, 0, 7.2, 71.9, 724, and 1457 mg/kg/day, respectively, and for females, 0, 8.1, 79.9, 791, and 1583 mg/kg/day, respectively) and fed to 10 F344 (Fischer) rats per sex per dose (plus 10 additional animals per sex at 0 and 20000 ppm to determine recovery) for 13 consecutive weeks (recovery group animals were observed for 4 additional weeks). No animals died. No clinical signs were observed. A treatment-related decrease in mean body weight was observed in both sexes at 10000 and 20000 ppm at Week 13, persisting in males at Week 17. Treatment-related decreases in mean red blood cell and hematocrit levels in males and mean hemoglobin level in both sexes at 10000 and 20000 ppm were observed at Week 13 with decreases persisting (except for the red blood cell parameter) in recovery group animals. Treatment-related increases in mean blood urea nitrogen (males), total bilirubin (males), total protein (males), γ-glutamyl transpeptidase (males and females), glutamic oxaloacetic transaminase (females) and alkaline phosphatase (males) levels were observed at 10000 and 20000 ppm at Week 13, with increases clearing (except for the blood urea nitrogen parameter) in recovery group animals. Treatment-related increases in mean relative liver, kidneys, spleen, and testes weights in males and in spleen weight in females were observed at 10000 and 20000 ppm at Week 13 with increases persisting in recovery group animals. Microscopic examination revealed numerous treatment-related effects including proliferative inter- and intralobular bile ducts in the liver (in both sexes at 10000 and 20000 ppm), fibrosis of the liver (in males at 10000 and 20000 ppm), sclerosis of the common bile duct (in males at 10000 and 20000 ppm and in females at 20000 ppm), and lymphocytosis in the urinary bladder (in both sexes at 10000 and 20000 ppm) at Week 13 persisting in recovery group animals. NOEL (M) = 71.9 mg/kg/day (1000 ppm) and NOEL (F) = 79.9 mg/kg/day (1000 ppm) (based on microscopic findings). **Acceptable**. (Corlett, 3/1/01)

055; 173505; "KIH-2023 Technical: A Four Week Dietary Range-Finding Study in Rats" (Inoue, H., Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shioshinden, Shizuoka-ken, Japan, Laboratory Project Identification: 1694, 9/5/91). KIH-2023 (Lot No. G35-01, purity = 96.3%) was admixed to the diet at dose levels of 0 (basal diet only), 100, 500, 20000, or 50000 ppm (for males, 0, 10.4, 51.4, 2078, and 5006 mg/kg/day, respectively, and for females, 0, 11.4, 56.6, 2208, and 5541 mg/kg/day, respectively) and fed to 10 F344 (Fischer) rats per sex per dose for 4 consecutive weeks. No animals died. Treatment-related wasting and piloerection were observed in both sexes at 50000 ppm. A treatment-related decrease in mean body weight was observed in males at 20000 and 50000 ppm and in females at 50000 ppm. Treatment-related decreases in mean hematocrit and hemoglobin levels were observed in both sexes at 20000 and 50000 ppm. Treatment-related increases in mean glutamic pyruvic transaminase and alkaline phosphatase levels were observed in both sexes at 20000 and 50000 ppm. A treatment-related increase in mean relative liver weights was observed in both sexes at 20000 and 50000 ppm. Necropsy revealed the following treatment-related effects: cecum with dilated lumen (in both sexes) and small testes at 20000 and 50000 ppm. Microscopic examination revealed treatment-related proliferative bile ducts in the liver in both sexes at 20000 and 50000 ppm. NOEL (M) = 51.4 mg/kg/day (500 ppm) and NOEL (F) = 56.6 mg/kg/day (500 ppm) (based on microscopic findings). **Supplemental** (test animals were treated for only 4 weeks and no ophthalmological examinations were conducted). (Corlett, 2/26/01)

057; 173507; "KIH-2023 Technical: Three Month Feeding Study in Mice" (Inoue, H., Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shioshinden, Shizuoka-ken, Japan, Laboratory Project Identification: 1929, 9/5/91). KIH-2023 (Lot No. G35-04, purity = 95.2%) was admixed to the diet at dose levels of 0 (basal diet only), 35, 350, 3500, or 7000 ppm (for males, 0, 6.8, 68.6, 699, and 1479 mg/kg/day, respectively, and for females, 0, 8.0, 79.0, 806, and 1591 mg/kg/day, respectively) and fed to 10 B6C3F_1 mice per sex per dose for 13 consecutive weeks. No animals died. No clinical signs were observed. A treatment-related decrease in mean body weight gain and a treatment-related increase in mean relative liver weight were observed in males at 7000 ppm. Microscopic examination revealed treatment-related swelling of liver cells at 3500 and 7000

ppm and epithelial hyperplasia of the gall bladder mucosa at 7000 ppm in both sexes. NOEL (M) = 68.6 mg/kg/day (350 ppm) and NOEL (F) = 79.0 mg/kg/day (350 ppm) (based on swelling of liver cells). **Supplemental** (no serum chemistry and no ophthalmological examinations were conducted on the test animals). (Corlett, 3/6/01)

064 173514 "KIH-2023: A Four-Week Dietary Range-Finding Study in Mice," (Inoue, H.; Biosafety Research Center, Foods, Drugs and Pesticides, (An-Pyo Center), Shizuoka-ken, Japan; Laboratory Project ID#: 1693; 9/5/91). KIH-2023 technical (96.3% pure) was fed in diet to B6C3F1 (SPF) mice (10/sex/dose) at 0, 10, 50, 500, 7000 and 30000 ppm for 4 weeks. NOEL = 50 ppm, based on male increased thymic karyorrhexia and decreased spleen weights and female decreased food efficiency at > 500 ppm. Other effects: Bodyweights and bodyweight gains were significantly decreased in females at $\geq$ 7000 ppm and in males at 30000 ppm. Males had decreased food efficiency at 30000 ppm. Both sexes had significantly decreased absolute brain, kidney, spleen and testes (male) weights at 30000 ppm. Males showed decreased relative kidney weights and increased brain weights at 30000 ppm. Females had significantly increased relative brain ($\geq$ 7000 ppm) and decreased spleen weights (30000 ppm). Both sexes had relative liver weight increases at 30000 ppm. Males had increased cecal dilated lumen at $\geq$ 7000 ppm. At 30000 ppm, males showed increased green gall bladder, black liver and white patches/zones on the liver. Females at 30000 ppm showed increased green gall bladder, black gall bladder, dilated cecal lumen and small uteri. Both sexes had increased liver and gall bladder effects at $\geq$ 7000 ppm. Females had increased stomach edema and thymic karyorrhexia at $\geq$ 7000 ppm. **No adverse effect**. These data are **supplemental**. M. Silva, 2/14/01.

059; 173509; "A Thirteen Week Oral Toxicity Study in Beagle Dogs with KIH-2023 Technical" (Kobayashi, K., Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shioshinden, Shizuoka-ken, Japan, Laboratory Project Identification: 2105, 3/4/92). 821. KIH-2023 (Lot No. G35-07, purity = 94.5%) was administered orally, via gelatin capsules, once daily to 4 beagle dogs per sex per dose at dose levels of 0 (empty capsule), 30, 100, or 600 mg/kg for 13 weeks. No animals died. Treatment-related vomiting, loose stool, and salivation were observed in both sexes at 600 mg/kg/day. Body weight, hematology, serum chemistry, urinalysis, ophthalmology, organ weight, and gross pathology investigations revealed no treatment-related effects. Microscopic examination revealed treatment-related proliferation of the bile ducts in the liver of males at 600 mg/kg/day. NOEL (M/F) = 100 mg/kg/day (based on clinical signs). **Acceptable**. (Corlett, 3/14/01)

058; 173508; "A Four Week Range-Finding Oral Toxicity Study in Beagle Dogs with KIH-2023 Technical" (Hashiguchi, J., Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shioshinden, Shizuoka-ken, Japan, Laboratory Project Identification: 1918, 3/22/91). KIH-2023 (Lot No. G35-04, purity = 95.2%) was administered orally using gelatin capsules daily to 2 beagle dogs per sex per dose at dose levels of 0 (empty capsule), 100, 300, or 600 mg/kg for 4 weeks. No animals died. Vomiting was observed in 1 female at 300 mg/kg/day and in 1 male and 2 females at 600 mg/kg/day. Loose stool was observed in 1 male at 300 mg/kg/day and in 1 male and 1 female at 600 mg/kg/day. Microscopic examination revealed proliferation of the bile ducts in the liver of all animals in both sexes at 300 and 600 mg/kg/day and epithelial hyperplasia in common bile duct of all males at 100, 300, and 600 mg/kg/day and of 1 female at 300 mg/kg/day, and of 2 females at 600 mg/kg/day. NOEL (M) = 100 mg/kg/day and (F) < 100 mg/kg/day (based on microscopic findings). **Supplemental** (only 2 animals per sex per dose were used, the animals were dosed for only 4 weeks, and no ophthalmological examinations were conducted on the test animals). (Corlett, 3/8/01)

060; 173510; "Three-day Preliminary Oral Toxicity Study in Beagle Dogs Treated with KIH-2023" (Hashiguchi, J., Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shioshinden, Shizuoka-ken, Japan, Laboratory Project Identification: 1978, 3/22/91). KIH-2023 (Lot No. G35-03, purity = 96.7%) was administered orally using gelatin capsules to 2 beagle dogs per sex. Each dog was administered a dose level of test article for 3 consecutive days followed by a recovery

period of 4 days and then was administered another dose level in the same manner. Each dog was administered consecutive dose levels 250, 500, 1000, and 750 mg/kg in this manner. No animals died. No clinical signs were observed when the animals were dosed with 250 and 500 mg/kg. Loose stool and vomiting were observed when the animals were treated with 1000 and 750 mg/kg (vomiting: both males and 1 females at 1000 mg/kg and 1 male and 1 female at 750 mg/kg; loose stool: 1 male and 1 female at 1000 mg/kg and 1 male at 750 mg/kg). All signs were observed during the dosing time intervals and not during the recovery time intervals. Macroscopic examination revealed no treatment-related abnormalities. NOEL (M/F) = 500 mg/kg/day (based on clinical signs). **Supplemental** (only 2 animals per sex were used, animals were dosed with multiple dose levels, animals were dosed only 12 times, and no hematology, no serum chemistry and no ophthalmological examinations were conducted on the test animals). (Corlett, 3/6/01)

(Dermal)

061; 173511; "21-Day Dermal Toxicity Study in Rats with KIH-2023 Technical" (Trutter, J.A., Corning Hazleton Incorporated (CHV), Vienna, VA, Laboratory Project Identification: CHV 2481-110, 1/25/96). 822. KIH-2023 TGAI (Lot No. G35-23-140, purity = 98.6%) was moistened with deionized water, poured onto a gauze pad, and applied to the clipped skin of 5 Sprague-Dawley Crl:CD®BR rats per sex per dose at dose levels of 0 (deionized water), 10, 100, or 1000 mg/kg for 6 hours daily for 21 consecutive days. No animals died. No treatment-related clinical signs or signs of dermal irritation were observed. Body weight determinations, hematology, serum chemistry, organ weight determinations, gross pathology, and histopathology revealed no treatment-related effects. NOEL (M/F, systemic and skin) = 1000 mg/kg/day (based on no effects at highest dose tested). **Acceptable**. (Corlett, 3/16/01)

METABOLISM STUDIES

091; 173441; "Study on the Metabolism of [^{14}C] KIH-2023 in Rats"; (P.E. Noker; Southern Research Institute, Birmingham, AL; Project ID No. SRI BIO-93-270; 12/29/93); Groups of Fischer 344 rats were dosed orally by gavage or by intravenous injection with bis-(pyrimidine-2-^{14}C) KIH-2023 (radiochemical purity: 98.3%, specific activity: 87.0 :Ci/mg) ([^{14}C] (Py) KIH-2023) or U-(benzene-^{14}C) KIH-2023 (radiochemical purity: >99%, specific activity: 50 :Ci/mg) ([^{14}C] (Bn) KIH-2023). In Groups 1A, B, C and D, 5 animals/sex/group were dosed orally with 30 mg/kg of either [^{14}C] (Py) KIH-2023 (A and C) or [^{14}C] (Bn) KIH-2023 (B and D). For Groups 2 A and B, 5 animals/sex/group were dosed daily by oral gavage with 30 mg/kg of KIH-2023, technical grade (purity: 97.2%, lot no. G35-13) for 14 days, followed a dose of 30 mg/kg of either [^{14}C] (Py) KIH-2023 (A) or [^{14}C] (Bn) KIH-2023 (B). For Groups 3A, B, C and D, 5 animals/sex/group were dosed orally by gavage with 600 mg/kg of either [^{14}C] (Py) KIH-2023 (A and C) or [^{14}C] (Bn) KIH-2023 (B and D). For Groups 4A, B, C and D, five animals/sex/group received intravenous injections of 30 mg/kg of either [^{14}C] (Py) KIH-2023 (A and C) or [^{14}C] (Bn) KIH-2023 (B and D). For all groups designated A or B, urine and feces samples were collected at 0-12 hr, 12-24 hr, 24-48 hr, 48-72 hr, 72-96 hr and 96-120 hours post-dose. At that time the animals were euthanized, a blood sample collected and the animals dissected for specified tissues. For all groups designated C or D, blood was collected from the tail vein at 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72 and 96 hours post-dose. At 120 hours, the animals were euthanized and a blood sample collected. The feces were the primary route of excretion for both of the radiolabeled compounds. At the lower dose, the males excreted from 11 to 13% and 80 to 85% of the administered dose in the urine and feces, respectively. At the higher dose, excretion in the urine ranged from 25 to 28% of the administered dose. In the feces, 70% of the dose was excreted. For the females, the percentage of administered dose excreted by either route did not vary at both dose levels. The % of administered dose excreted in the urine ranged from 28 to 37%. For the feces, the range was from 48 to 60% (note: two of the radiolabel recovery values from the feces, for Groups 1A and 2A, were close to or in excess of 100% of the administered dose and appeared to be at variance from all of the other data collected). At the lower dosing level via the oral route, peak plasma levels were achieved within 30 minutes of dosing for both radiolabeled compounds. At 600 mg/kg, the plasma levels remained at a peak concentration for up to 4 hours post-dose. The pharmacokinetic parameters

(calculated for only the 30 mg/kg doses) were as follows: males, $t_{1/2}$-28.4 to 37.9 hours, t_{max}-0.29 to 0.31 hours, females, $t_{1/2}$-28.0 to 37.1 hours, t_{max}-0.32 to 0.36 hours. The liver, intestinal tract and plasma were the primary sites of recovery of radiolabel at 120 hours post-dose. Analysis of the radiolabeled material revealed the unmetabolized test compound was the primary moiety excreted. This moiety constituted 68.5 to 82.9% of the administered dose recovered from both the feces and urine (note: the value for the 3B females of 99.8% was well in excess of all of the other recoveries). The quantity of administered dose, sex of the treated animal or the route of treatment did not greatly alter the % of unmetabolized test material recovered. The test material was metabolized by cleavage of the ether linkage between the benzene and pyrimidine rings and removal of the methyl groups from the pyrimidine moieties. The position of the radiolabel did not alter the metabolic profile. **Study supplemental.** (Moore, 5/29/01)

52841-092; 173442; "Determination of Bile Acids in Serum after Two-Week Consecutive Oral Administration of KIH-2023 by Feed-admixture to Male Mice"; (Yuki, K.; Panapharm Laboratories Co., Ltd., Safety Assessment Laboratory, Uto-shi, Kumamoto 869-04, Japan; Project ID No. PPL99411; 6/27/95); Ten B6C3F1 male mice/group were fed 0 or 7000 ppm of KIH-2023 Technical (lot no. G35-13-115; purity: 95.0%) in the diet for 14 days (0 or 1290 mg/kg/day). The treated animals suffered no deaths or treatment-related clinical signs. Nine of the 10 treated animals exhibited a slight enlargement of the cecum. No treatment-related effect was noted on the absolute or relative liver weights. The total bile acid content in the serum was increased for the treated animals (7000: 3.44 vs. 0:1.60 nmol/ml). **Supplemental Study**. (Moore, 6/12/01)

52841-093; 173443; "Determination of Bile Acids in Serum after Two-Week Consecutive Oral Administration of KIH-2023 by Feed-admixture to Male Rats"; (Yuki, K.; Panapharm Laboratories Co., Ltd., Safety Assessment Laboratory, Uto-shi, Kumamoto 869-04, Japan; Project ID No. PPL99412; 6/27/95); Five male F344/Du Crj rats/group received 0 or 20000 ppm of KIH-2023 Technical (lot no. G35-13-115; purity: 95.0%) in the diet for 14 days (0 or 1792 mg/kg/day). No mortality resulted from the treatment. The mean body weight for the treated animals was less than that of the controls after two weeks of treatment ($p<0.05$). Likewise, mean food consumption was less than that of the controls ($p<0.05$). Slight enlargement of the cecum was noted for all of the treated animals. No treatment-related effect on the mean absolute or relative liver weights was evident. The mean serum concentration of total bile acids was increased for the treated animals above that of the control animals (20000: 40.88 vs. 0:3.49 nmol/ml) ($p<0.05$). This increase was reflected in elevated levels of glycocholic, taurocholic and deoxycholic acid in the serum. **Supplemental Study.** (Moore, 6/12/01)

088; 173438; "Preliminary Study on the Absorption, Distribution, Metabolism and Excretion of [^{14}C KIH-2023 in Rats"; (P.E. Noker; Southern Research Institute, Birmingham, AL; Project ID No. SRI-BIO-90-573; 6/21/91); In phases A and D, One Fischer 344 rat/sex/group was dosed by either oral gavage or intravenous injection with 100 mg/kg of [^{14}C] KIH-2023 (specific activity: 1.50 GBq/mmol, radiochemical purity: 98.2%) (label on the 2nd carbon of the pyrimidine ring). Technical grade KIH-2023 (purity: 95.2%) was used to supplement the dosing preparations. Carbon dioxide was collected up to 24 hours post-dose. Urine and feces were collected at the 0-12, 12-24, 24-48 and 48-72 hour intervals. In phase B, two rats/sex were dosed by oral gavage with 100 mg/kg of the test material. At 15 and 30 minutes and 1, 2, 4, 8, 24, and 48 hours post-dose, blood was collected by retroorbital bleeding from one male and one female. In phase C, two rats/sex were dosed by oral gavage with 100 mg/kg of the test material. One animal/sex was euthanized by exsanguination at 2 and 72 hours post-dose. Blood was collected along with specified tissues. In phase E, one male was treated by oral gavage with 100 mg/kg of the test material. Urine and feces were collected over 0-24 and 24-48 hour intervals post-dose for metabolite analysis. When the test material was dosed orally, a greater percentage of the dose was recovered in the feces. Intravenous injection resulted in a lower percentage of radiolabel recovered in the feces with a corresponding increase in recovery from the urine. The presence of the radiolabel in the feces after intravenous dosing indicated that biliary excretion had occurred. Recovery of radiolabeled carbon dioxide was negligible. Peak blood levels were evident at 2 hours post-dose. The highest levels of radiolabel recovered in the 2 hour samples were in the contents of the gastrointestinal tract. Otherwise, the level of radiolabeling in the tissues had largely dissipated by 72 hours. The predominant radioalabeled moiety in both the urine and feces was the parent compound. It constituted 86% of the recovered label. Demethylation of the parent compound was the predominant metabolic process identified. **Study supplemental.** (Moore, 5/30/01)

089; 173439; "Preliminary Study on the Absorption, Distribution, Metabolism and Excretion of [^{14}C] KIH-2023 in Rats (Addendum 1 to Final Report SRI-BIO-90-573)"; (P.E. Noker; Southern Research Institute, Birmingham, AL; Project ID. No. SRI-BIO-91-747; 2/26/92); Additional analysis was performed on the radiolabeled compounds recovered in the urine collected at the 0-24 and 24-48 hour intervals from a male Fischer 344 rat treated orally by gavage with 100 mg/kg of [^{14}C] KIH-2023 (specific activity: 1.50 GBq/mmol, radiochemical purity: 98.2%) (label on the 2nd carbon of the pyrimidine ring). The second analysis was performed using additional standards in order to determine the identity of particular chromatographic peaks not previously ascertained. The results of the reanalysis were essentially the same as those noted in the original report. **Study supplemental.** (Moore, 6/1/01)

090; 173440; "Study on the Biliary Excretion of [^{14}C] (Py) KIH-2023 in Rats"; (P.E. Noker; Southern Research Institute, Birmingham, AL; Project ID No. SRI-BIO-92-049; 3/31/94); Five Fischer 344 rats/sex/group were dosed orally with either 10 or 100 mg/kg of [^{14}C] (Py) KIH-2023 (specific activity: 1.46 GBq/mmol, radiochemical purity: 97.7%, lot no. CP-1221) following overnight fasting. KIH-2023 technical (purity: 95.2%, lot no. G35-04) was used in the preparation of the dosing solutions. Bile was collected hourly through the first 6 hours post-dose and at the 6-12 and 12-24 hour intervals. Urine and feces were collected at 0-6, 6-12 and 12-24 hour intervals post-dose. The percentage of the administered dose recovered in the bile over the first 24 hours after dosing was approximately 36 to 37% for the males and 24 to 27% for the females at the two dose levels. In that first 24 hours, 2.4% or less of the dose was recovered in the feces. However, at 24 hours, 22.5 to 30.0% of the radiolabel at the low dose and 40 to 44% at the high dose was still associated with the tissues of the gastrointestinal tract and may not have been absorbed. Only two of the five major radiolabeled moieties in the bile were identified, demethylated KIH-2023 and KIH-2023. These two moieties constituted 52.0 to 60.1% of the recovered radiolabel in the bile. Overall, the data indicate that the hepato-biliary pathway is a significant route for the uptake and excretion of the test material. **Study supplemental.** (Moore, 5/30/01)

Collectively, these studies fulfill the requirements for metabolism study; single low dose, single high dose, 14-day repeated low dose and intravenous dosing at the low dose.